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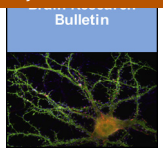

Research report

Lifelong consumption of *trans* fatty acids promotes striatal impairments on Na^+/K^+ ATPase activity and BDNF mRNA expression in an animal model of mania

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ABSTRACT

Purpose: To evaluate the toxicity of chronic consumption of processed foods that are rich in *trans* fat on the lipid composition of brain membranes, as well as its functional repercussions.
Methods: A second generation of male rats born from mothers and grandmothers supplemented with soybean oil (SO—C, an isocaloric control group) or hydrogenated vegetable fat (HVF, rich in TFA) (3 g/kg; p.o.) were kept under oral treatment until 90 days of age, when they were exposed to an AMPH-induced model of mania.
Results: The HVF group presented 0.38% of TFA incorporation in the striatum, affecting Na^+/K^+ ATPase activity, which was decreased per se and following AMPH-exposure. The HVF group also showed increased protein carbonyl (PC) and brain-derived neurotrophic factor (BDNF) mRNA levels after AMPH administration, while these oxidative and molecular changes were not observed in the other experimental groups. Additionally, a negative correlation between striatal Na^+/K^+ ATPase activity and PC levels ($r^2 = 0.49$) was observed.
Conclusion: The prolonged consumption of *trans* fat allows TFA incorporation and increases striatal oxidative status, thus impairing the functionality of Na^+/K^+-ATPase and affecting molecular targets as BDNF mRNA. We hypothesized that the chronic intake of processed foods (rich in TFA) facilitates the development of neuropsychiatric diseases, particularly bipolar disorder.

1. Introduction

Literature has shown a vital influence of the diet during the gestational and neonatal periods, whose content may exert a prolonged influence on brain health status (Anjos et al., 2013). Nowadays, several efforts have been made to reduce and/or eliminate *trans* fatty acids (TFA) from foods in several countries (Remig et al., 2010; Mozaffarian, 2010; Downs et al., 2013), mainly due to their considerable influence on the development of cardiovascular diseases. From this point of view, it is important to know that *trans* fat consumption, even in small amounts, is able to exert long-term deleterious influences, also affecting the functioning of the central nervous system (CNS) in addition to the peripheral damage already described. In fact, recent studies of our group have shown significant impairments in the central nervous system (CNS) caused by trans fat (Trevizol et al., 2013, 2015a,b; Kuhn et al., 2015; Pase et al., 2015; Dias et al., 2015). This Western eating habit favors brain incorporation of TFA in the neural membranes, thus facilitating the development of neuropsychiatric conditions (Trevizol et al., 2015a,b; Dias et al., 2015) and its consequent incorporation in the CNS is currently a matter of concern in public health.

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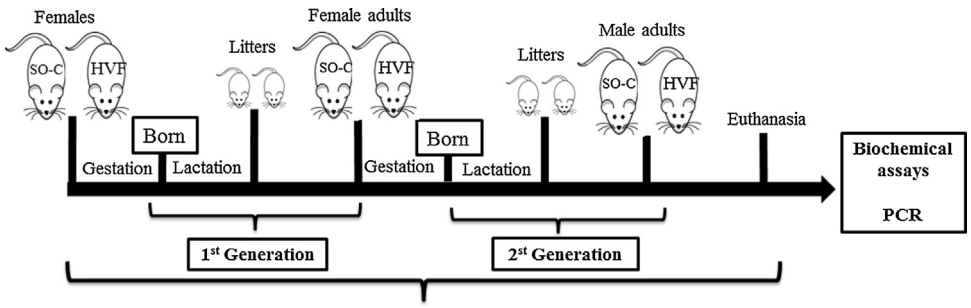

Fig. 1. Experimental design. Abbreviations: SO—C: soybean oil-control; HVF: hydrogenated vegetable fat.

Bipolar disorder (BD) is a highly debilitating disease (Merikangas et al., 2011) and there is an increased need for research into its etiology, which remains unknown. While the hippocampus and prefrontal cortex have been widely studied as brain areas potentially affected by BD, few studies considered the striatum in this condition. A recent clinical study (Mason et al., 2014) associated ventral-fronto-striatal areas with impulsivity and risk decision-making in BD patients, while striatal activation has been implicated in the hedonic impact of risk and reward. Furthermore, changes in intracellular pathways related to oxidative status, neuronal transmission and plasticity have been linked to neuropsychiatric diseases, including BD (Mason et al., 2014).

In this line of evidence, as fatty acids (FA) are vital to brain membranes, thus affecting their morphology, neurophysiology and neurotransmission (Anjos et al., 2013), we assumed that the presence of TFA in the brain neural membranes could affect those pathways. Indeed, here we have shown for the first time that dietary *trans* fat supplementation during two sequential generations of animals was able to cause oxidative, functional and molecular impairments in the striatum, following an animal model of mania.

2. Material and methods

All animal procedures were approved by the Ethics Committee of Animal Use of the Federal University of Santa Maria. Rats were kept in Plexiglas cages with access to food and water ad libitum in a room with controlled temperature. To perform this trial, second generation male Wistar rats were orally supplemented with soybean oil (SO—C, isocaloric control group) or HVF (rich in TFA) until 90 days of age, when they were submitted to an amphetamine (AMPH)-induced model of mania (Fig. 1). The detailed experimental protocols and the fatty acids (FA) profile of each supplementation were described elsewhere (Trevizol et al., 2015a). Animals were anesthetized (sodium pentobarbital, 50 mg/kg body weight ip) and euthanized by cervical decapitation. The striatum was dissected out for assessment of FA profile (Trevizol et al., 2015b), protein carbonyl (PC) levels estimation (Trevizol et al., 2015a), Na^+/K^+ ATPase activity (Muszbek et al., 1977) and BDNF (brain-derived neutrophic factor) mRNA expression (Trevizol et al., 2015b). Results are presented as mean ± S.E.M. and analyzed by two-way ANOVA followed by Duncan's post hoc test. The significance level was set as $p < 0.05$.

3. Results

As shown in Table 1, an incorporation of C18:1 *n*-9t, totaling 0.38% ΣTFA in the striatum, was found exclusively in the HVF group. In addition, HVF supplementation increased ΣSFA and decreased ΣMUFA in relation to SO—C group.

Table 1
Fatty acid composition of second generation of rat's striatum supplemented with different oil/fat. (% Of total fatty acids identified).

Fatty acid	Mean (±SEM) SO—C	HVF
C16:0	17.37 ± 0.45	19.01 ± 0.42*
C18:0	19.02 ± 0.34	19.53 ± 0.25
∑ SFA	40.60 ± 0.67	43.09 ± 0.98*
C16:1 *n*-7	0.66 ± 0.05	0.72 ± 0.05
C18:1 *n*-9	18.44 ± 0.65	20.10 ± 0.75
C18:1 *n*-7	4.16 ± 0.33	3.18 ± 0.31*
C18:1 *n*-9t	n.d.	0.38 ± 0.08*
C20:1 *n*-9	3.42 ± 0.49	2.49 ± 0.09
∑ MUFA	36.59 ± 0.67	33.82 ± 0.58*
C18:2 *n*-6	1.96 ± 0.11	2.06 ± 0.21
C20:4 *n*-6	7.76 ± 0.16	8.16 ± 0.12
C22:4 *n*-6	3.26 ± 0.16	3.20 ± 0.20
C22:5 *n*-6	0.55 ± 0.02	0.78 ± 0.05*
C22:5 *n*-3	0.14 ± 0.00	0.11 ± 0.00*
C22:6 *n*-3	8.37 ± 0.22	8.31 ± 0.02
∑ PUFA	22.80 ± 0.39	23.52 ± 0.09
∑ *n*-3	8.55 ± 0.20	8.68 ± 0.06
∑ *n*-6	14.20 ± 0.24	14.65 ± 0.32
∑ TFA	n.d	0.38 ± 0.08*
n-6/*n*-3 ratio	1.66 ± 0.03	1.68 ± 0.03

The following fatty acids were found at concentrations lower than 0.5% and for this reason are not shown: C18:2n6t, C18:3n6, C21:0, C20:3n3, C20:5n3, C22:2n6, C23:0. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: trans fatty acids.

* Indicate significant difference between SO—C and HVF group ($P < 0.05$).

Post-hoc test showed that the AMPH administration significantly increased PC levels in HVF group compared to SO—C group (Fig. 2).

HVF-supplemented animals presented decreased Na^+/K^+ ATPase activity per se and following AMPH administration in relation to SO—C group, while AMPH administration reduced the enzyme activity only in HVF group (Fig. 2).

Post-hoc test revealed that AMPH administration increased striatal BDNF mRNA expression only in HVF group (Fig. 2). Additional analyses revealed a significant negative correlation between Na^+/K^+ ATPase activity and PC levels (Fig. 3).

4. Discussion

The brain undergoes continuous structural remodeling in response to signals originating from inside and outside of the body. During the developmental period, brain areas are drastically modified by dietary content, which may impair their physiologic functions. In view of this, the unknown etiology of neuropsychiatric

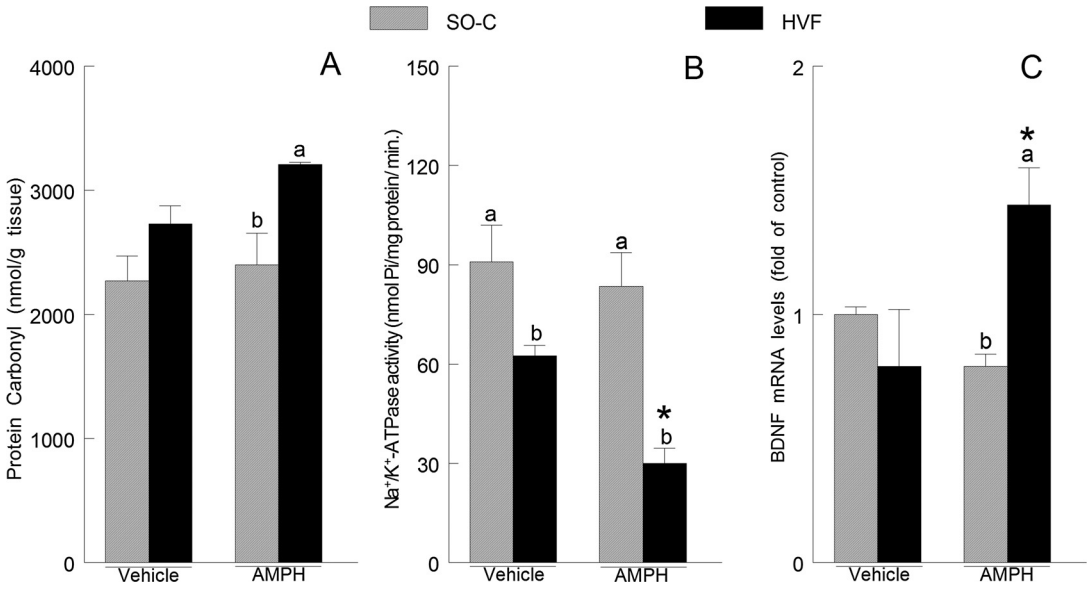

Fig. 2. Striatal protein carbonyl levels estimation (A), Na^+/K^+ ATPase activity (B) and BDNF mRNA expression (C) of a second generation of rats supplemented with SO—C or HVF until adulthood when they were injected with AMPH (4 mg/kg) or vehicle for 14 days. Data are expressed as mean ± S.E.M. Abbreviations: SO—C: soybean oil-control; HVF: hydrogenated vegetable fat. Different lowercase letters indicate differences between supplementations in the same drug treatment ($P < 0.05$). * Indicates significant differences from vehicle in the same supplementation ($P < 0.05$).

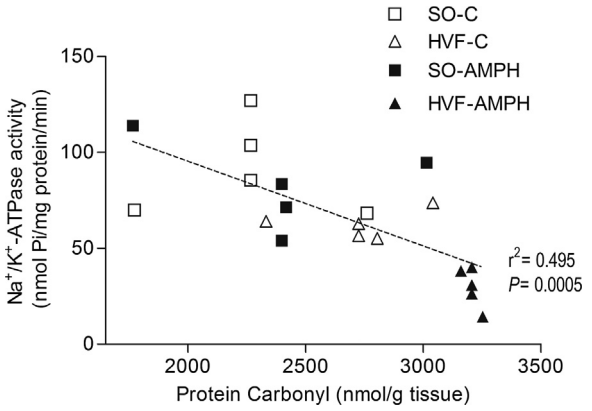

Fig. 3. Correlation analysis between Na^+/K^+ ATPase activity and PC levels estimation in striatum of rats of a second generation supplemented with SO—C or HVF until 90 days of age, when they were injected with AMPH (4 mg/kg) or vehicle for 14 days. Statistical analysis revealed the following $P = 0.0005$ significance levels for the $r^2 = 0.49$ value.

disorders could be related, at least in part, to bad dietary habits, as most of the lipid content is concentrated in the neuronal membrane phospholipids.

TFA, which are present in processed foods, have become a source of concern due to their brain incorporation, which has been associated to neuropsychiatric damage, as observed in animals of first generation (Trevizol et al., 2013). In order to assess long-term brain impairments, additional studies were performed with animals from second generation (Trevizol et al., 2015a,b). In the present study we are showing TFA effects on the striatum since this brain area is related as well to bipolar disorder and other neuropsychiatric disorders (Merikangas et al., 2011; Suarez et al., 2014).

Our findings showed a significant incorporation of 0.38% of C18:1 *n*-9t (*trans* elaidic acid) in the striatum of HVF-supplemented rats. We believe that such presence could be a key factor to the impairments observed here, such as decreased activity of Na^+/K^+ ATPase in striatum. In fact, this membrane-bound enzyme regulates the ionic gradient in brain cell membranes directly involved in BD-related neuronal transmission (Srinivasarao et al., 1997). In line with our findings, a higher fluidity of neural membranes, which has been related to increased unsaturated/saturated ratio, has been pointed as responsible for optimal Na^+/K^+ ATPase activity (Srinivasarao et al., 1997). On the other hand, HVF supplementation showed an inverse influence in these parameters, enabling us to infer that TFA may reduce phospholipid membrane fluidity in the striatum. Reinforcing these findings, we found a negative correlation between Na^+/K^+ ATPase activity and PC levels, which is a recognized oxidative damage marker. We can infer a close relationship due to altered membrane fluidity with oxidative stress and changes in neuronal transmission.

Considering neuronal plasticity, BDNF is a neurotrophin involved in this mechanism, thus related to BD as well. The binding of mature BDNF to the tropomyosin kinase receptor (TrkB) is responsible for beneficial influences, such as synaptic efficiency, neuronal connectivity and neuroplasticity (Grande et al., 2011). In contrast, the pro-form of this neurotrophin (proBDNF) preferentially binds to the pan-neurotrophin receptor ($p75^{NTR}$), which mediates apoptotic mechanisms (Grande et al., 2011). At the molecular level, we observed an increase in BDNF mRNA expression in the HVF group after AMPH-exposure. This finding is in agreement with McGinty et al. (2011), who demonstrated that psychostimulant drugs are able to increase BDNF mRNA in the striatum trying to bring the network with dopamine back to homeostasis. From these findings, we may infer that this AMPH-induced molecular change in the HVF group is related to transcriptional changes, which could modify neurotrophins synthesis with consequent apoptosis development.

This is the first study which shows the relationship between TFA supplementation and BDNF mRNA expression in striatum of rats from second generation. Furthermore, we can infer that different brain areas react differently to the challenge of having TFA in neural membranes (Trevizol et al., 2015a,b). In our study, only after AMPH-exposure we were able to observe changes in BDNF mRNA expression in HVF group. We believe that adaptive metabolic processes may be activated in HVF-supplemented animals when this fat is received from gestation followed by two sequential generations.

5. Conclusion

In summary, we observed that TFA from processed foods might be significantly incorporated into striatum across generations, indicating that this type of FA may be modifying the striatal membranes causing impairments in BD-related pathways.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgments

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